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Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat

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Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. We have followed the concentration and fractional composition of tubular fluid protein and urinary protein in spontaneously hypertensive rats (SHR) from 6 to 65 weeks of their age. At intervals these functional data were correlated with the morphologic changes read by light microscopy. Matched control animals were of the same genetic strain, Wistar-Kyoto (WKY). Proximal tubular fluid protein was measured by capillary polyacrylamide gel electrophoresis; urinary protein, by Lowry method; and fractionation, by gradient gel electrophoresis. Tubular fluid protein in superficial nephrons did not change, although protein excretion rose in the SHR group to six times the control value (4.3 to 26 mg/24 hr × 100 g of body wt). The albumin fraction of urinary protein increased in the SHR from 10.5 ± 3.1% (SD) at week 6 to 72.3 ± 9.0% at week 65. No change occurred in WKY controls. Pathologic changes in SHR were strikingly limited to glomeruli and vessels of inner cortical nephrons, and progressed rapidly from 45 to 65 weeks. Glomeruli showed focal sclerosis with obliteration of the capillary tuft. Corresponding tubules were filled with protein casts. Small arteries and arterioles showed thickening and hyperplasia, particularly in the medial layer. Periglomerular fibrosis was moderate to marked. None of these changes were found in normotensive controls. The distinguishing pattern of proteinuria (albuminuria coincident with a decrease in low mol wt protein excretion) which evolved during the first year in SHR was temporally related to glomerular and arteriolarsclerotic changes. These changes mainly affected the deep cortical nephrons, which were less protected, perhaps, by autoregulation in the face of sustained hypertension. Superficial glomeruli were initially spared, and this is consistent with the finding of normal tubular fluid protein concentration in surface nephrons.

Sélectivité des lésions rénales et de la protéinurie chez les rats spontanément hypertendus. Nous avons étudié le débit et la composition de la protéinurie chez les rats spontanément hypertendus (SHR) de la sixième à la soixante cinquième semaine de vie. Les résultats des études fonctionnelles ont été corrélés, à intervalles réguliers, aux modificiations observées en microscopie photonique. Les contrôles ont été des animaux de la même souche génétique, Wistar-Kyoto (WKY). La concentration de protéine dans le liquide tubulaire proximal a été mesurée par électrophorése sur gel de polyacrylamide, dans l'urine par la méthode de Lowry et le fractionnement par électrophorèse en gradient de gel. La concentration de protéine dans les néphrons superficiels n'augmente pas alors que l'excrétion de protéine augmente pour atteindre six fois la valeur contrôle dans le groupe SHR (4,3 à 26 mg/24 hr × 100 g poids corporel). La fraction d'albumine de la protéinurie augmente chez le SHR de 10,5 ± 3,1% (SD) à 6 semaines jusqu'à 72,3 ± 9,0% à 65 semaines. Aucune modification n'apparaît chez les contrôles WKY. Les modifications histologiques chez les SHR sont limitées, de façon frappante, aux glomérules et aux vaisseaux des néphrons du cortex profond. Elles progressent rapidement de 45 à 65 semaines. Les glomérules ont une sclérose focale avec oblitération du capillaire. Les tubes correspondants sont remplis de cylindres protéiques. Les petites artères et les artérioles sont épaissies et hyperplasiques, en particulier aux dépends de la couche moyenne. La fibrose péri-glomérulaire est au moins modérée. Aucune de ces modifications n'est observée chez les contrôles à pression artérielle normale. La modalité particulière de la protéinurie (albuminurie qui coincide avec une diminution de l'excrétion des protéines de faible PM) qui se développe pendant la première année chez les SHR est liée dans le temps aux modifications glomérulaires et vasculaires. Ces modifications intéressent surtout les néphrons profonds, qui sont peut-être moins protégés, par l'autorégulation, d'une hypertension prolongée. Les glomérules superficiels sont initialement épargnés et ceci explique que l'on trouve une concentration normale de protéine dans le liquide tubulaire des néphrons superficiels.

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In human hypertension, proteinuria may constitute a significant urinary abnormality, usually associated with the late stages of the disease [1]. The Framingham Heart Study [2] found albuminuria to be an important prognostic sign, being associated with a three-fold increase in mortality from cardiovascular complications of hypertension.

We have examined the relationship between proteinuria and morbidity in an extended study of the spontaneously hypertensive rat, using a matched normotensive group of the same genetic strain as controls. Both groups were followed from the 6th to 65th week of life, documenting the natural history of proteinuria, as well as a record of renal function and the morphologic changes in blood vessels and architecture of the kidney as seen by light microscopy.

Methods

Male rats used in this study were of the Wistar-Okamoto spontaneous hypertensive (SHR) strain (*N*

= 51) and the Wistar-Kyoto (WKY) normotensive strain (N = 59), both provided by the Laboratory Supply Company, Indianapolis, IN. The hypertensive and normotensive rats were F-generation 35 and 12, respectively. At least one week was allowed for the animals to adapt to our facilities before any experimental procedures were performed. All animals had free access to food and water, and no difference in intake was observed between experimental and control groups. A reduction in the number of rats under study occurred as animals from each group were used for acute micropuncture experiments.

Blood pressure measurements. Indirect systolic blood pressure was determined by the tail-cuff method without anesthesia, the animals soon becoming accustomed to this procedure. The average of at least three readings, taken in a quiescent state, was recorded.

Direct blood pressure measurements were obtained on anesthetized animals by carotid artery catheterization with a 19–gauge low compliance catheter which was filled with heparinized saline solution and connected to a transducer (Statham P23Gc) attached to a polygraph (Grass, model 79). The mean systolic and diastolic pressures were taken as the average of at least three consecutive readings. Mean pressures were calculated by adding one-third of the pulse pressure to the diastolic pressure [3].

Weights. Body weights were recorded to the nearest gram by use of a triple beam balance (Ohaus Scale Corp., Union, NJ). Heart and kidneys from each experimental animal were immersed briefly in 10% phosphate buffered formalin and then weighed on a Mettler balance to the nearest tenth of a gram. Urine volumes were equated with urine weight.

Operative procedures. The micropuncture procedure followed the standard usage of this laboratory [4]. A venous infusion of isotonic saline solution with synthetic inulin (4 g/100 ml of Laevosan) and para-aminohippuric acid (PAH, 0.2 g/100 ml) was maintained at a rate of 0.06 ml/min for the determination of glomerular filtration rate (GFR), proximal tubular fluid to plasma (TF/P) inulin (In) ratios, and effective renal plasma flow.

At the start of each clearance period, a bladder and/or ureteral catheter was led into a weighed test tube for urine collection, and tail vein blood was collected into heparinized capillary tubes.

Collection micropipettes (tip diameter, 8 to 10 μ), filled just before use with castor oil dyed with Sudan Black B, were positioned over the kidney surface with a micromanipulator (Leitz). Proximal tubular collections of 20 to 40 nl were made under free-flow conditions over two- to six-minute periods. A drop of oil expelled from the collection pipette indicated the direction of flow and identified the end proximal portion of the tubule. Each hour, blood samples were taken, and in experiments longer than three hours, two or more bladder urine samples were collected. At the conclusion of each experiment, the heart and kidneys were removed and fixed in 10% phosphate-buffered formalin.

Analytical methods. Urinary and plasma inulin were analyzed by the Anthrone method of Fuhr, Kaczmarczyk, and Kruttgen [5]; tubule fluid inulin, by a micromodification of the same method [6]. Color development in the microsamples was read in a spectrophotometer (Gilford) adapted for a 3-μl cuvette. PAH concentrations in urine and plasma were analyzed according to the method of Smith et al [7].

Total protein concentration in overnight urine was determined by the method of Lowry et al [8] after trichloroacetic acid precipitation. Total plasma protein was determined by a Biuret method [9]; and albumin concentration, by electrophoretic analysis (see following). Urine and plasma creatinine were analyzed after adsorption and elution from Lloyd's reagent [10].

Analysis of protein in proximal tubular fluid was by a modification [11] of Oken's technique for capillary-contained polyacrylamide gel electrophoresis [12]. Sensitivity of the method is such as to detect 10^{-10} g of albumin in the sample. Complete descriptions of the method have been published [11, 12]. Tubule fluid samples were transferred directly from the collection pipet to an analytical capillary tube within one hour of collection. Sample volume was determined by length of the column, as measured by an eyepiece micrometer. Appropriate standards of diluted rat serum in volumes comparable to unknowns supplied reference curves for each experiment.

A micro-continuous gradient gel electrophoresis procedure was used for separation and quantitation of proteins in plasma (diluted 1:101) and urine [13]. The continuous gradient gels were made in 5-μl microcaps. Solutions of acrylamide, buffer, and ammonium persulfate were added to the microcaps to provide a gradient of 4 to 40% polyacrylamide. Samples were added to the gel in a manner similar to that used for the discontinuous method. In contrast to the disc procedure, however, the top 75 nl of the non-polymerized acrylamide in the microcap were drawn off and replaced by an equivalent amount of sample or standard. The remaining empty portion of the microcap was filled with buffer, and electropho-

resis was run for forty minutes. The gels were extruded from the capillary tubes, stained for at least two hours, destained overnight, and read in an ultramicrodensitometer (Joyce-Loebl) with an integrator unit.

Tissue preparation. The kidneys and heart were fixed in 10% phosphate-buffered formalin and embedded in paraffin for light microscopic studies. Fixed tissues were processed in an Autotechnicon in graduated alcohol solutions and vacuum-dried under fifteen pounds of pressure. Sections were cut at thicknesses of 4 to 6 μm and stained according to the *Armed Forces Institute of Pathology Manual* (1968) with hematoxylin-eosin, periodic acid Schiff (PAS), Gomori's aldehyde fuchsin, and Masson's trichrome methods.

Results

Body and organ weights. Body weight was not conspicuously different in the control and hypertensive groups. Except for a brief period (weeks 9 through 13), the WKY rats tended to be heavier than the SHR's. From week 37 on, the SHR's weights leveled off, whereas their normotensive controls continued to gain normally. This resulted in a significant difference in weight at week 65 ($P < 0.01$).

Kidney weight as a fraction of body weight was not different in the two groups, but heart weights of SHR's, so expressed, were significantly greater after week 30 ($P < 0.001$) (Fig. 1). Divergence of the regression lines describing the heart weight ratios is exaggerated by the failure of SHR's to increase their body weight after the 37th week.

Blood pressure measurements. The systolic blood pressure of the control group, as measured by the indirect tail-cuff method without anesthesia, averaged 127.6 ± 5.2 (SD) mm Hg from week 6 to 65 (Fig. 2). Blood pressures of the SHR reached hypertensive

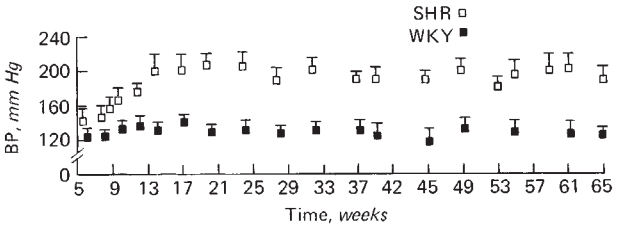


Fig. 2. *Blood pressure vs. age.* The blood pressure is systolic determined by indirect methods without anesthesia. The *vertical T-bars* denote SD. WKY, $N = 16$; SHR, $N = 20$.

levels (above 150 mm Hg) at week 9, continued to increase to week 13, and remained elevated (199.0 ± 8.1 (SD) mm Hg) with minor variations throughout the study (Fig. 2).

Systolic blood pressures were not significantly different whether measured directly or indirectly, with or without anesthesia. It is interesting that pulse pressures were quite similar in the experimental and control groups. In four animals of each group, direct readings gave values of 107/57 ± 9.45/6.45 and 226/175 ± 10/8 mm Hg for WKY's and SHR's, respectively. Mean arterial pressures were 76.3 ± 5.9 and 192.8 ± 8.8 mm Hg, and heart rates were 360 and 420 beats/min for these WKY's and SHR's, respectively.

Renal function. Creatinine clearance (ml/min × 100 g of body wt) was determined from overnight

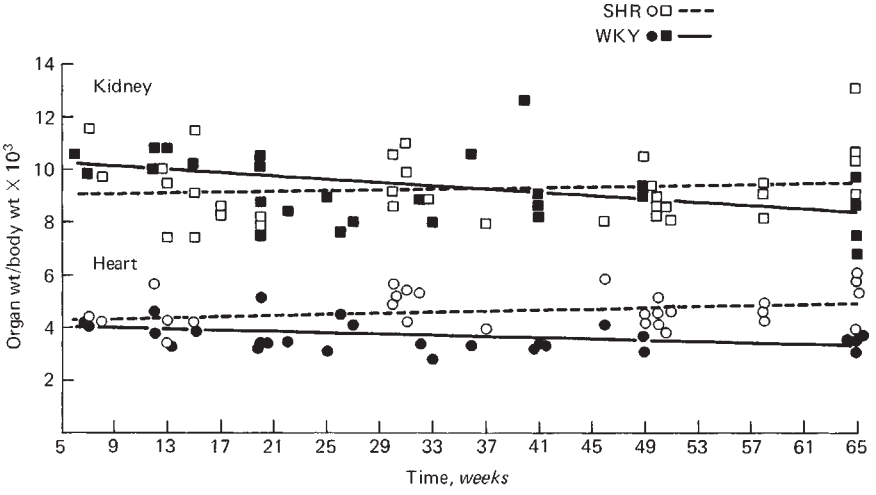


Fig. 1. *Organ weight as a function of body wt vs. age.* The equations for the regression lines are the following. *Kidney weight:* WKY − y = −0.03x + 10.18 ($r = 0.41$, $N = 27$) and SHR −y = 0.006x + 9.04 ($r = 0.09$, $N = 25$). *Heart weight:* WKY − y = −0.012x + 4.03 ($r = 0.41$, $N = 27$) and SHR − y = 0.008x + 4.33 ($r = 0.21$, $N = 25$).

urine collection (Table 1). Clearance values in the WKY group did not change significantly from weeks 8 to 65. The SHR showed a significant decrease in

Table 1. Clearance and plasma concentration of creatinine WKY and SHR strains of rats[a]

	Weeks	Mean	SD	No. of clearance periods	*P*
Creatinine clearance, *ml/min* × 100 *g of body wt*					
WKY	8–65	0.54	0.08	108	—
SHR	8–43	0.56	0.06	51	NS
	50–63	0.41	0.04	33	<0.001
	65	0.34	0.16	6	<0.01
Plasma creatinine, *mg/100 ml*					
WKY	8–65	0.40	0.07	108	—
SHR	8–43	0.46	0.12	51	NS
	50–63	0.55	0.13	53	<0.05
	65	0.64	0.14	6	<0.001

[a] Data obtained from overnight urine collections

the 50 to 63 week group ($P < 0.001$) and again at the 65th week ($P < 0.01$). Plasma creatinine concentration (mg/100 ml) of WKY rats was stable over the 8 to 65 week period (Table 1). Values for the SHR group showed a progressive increase with age. They were significantly higher than controls from week 50 on ($P < 0.05$).

In Table 2 are recorded mean urine flows, filtration

Table 2. Renal function in WKY and SHR rats[a]

	Weeks	Mean	SD	No. of rats	*P*
Mean urine flow, *ml/min* × *g of kidney wt*					
WKY	7–65	2.49	0.70	27	—
SHR	7–58	2.87	1.46	27	NS
	65	2.70	0.90	4	NS
GFR, *ml/min* × *g of kidney wt*					
WKY	7–65	0.57	0.25	27	—
SHR	7–58	0.53	0.27	27	NS
	65	0.30	0.14	4	<0.005
C_{PAH}, *ml/min* × *g of kidney wt*					
WKY	7–65	2.09	0.86	26	—
SHR	7–58	1.41	0.74	26	<0.005
	65	0.99	0.52	4	<0.005
Filtration fraction					
WKY	7–65	0.30	0.09	26	—
SHR	7–58	0.37	0.09	26	<0.025
	65	0.31	0.05	4	NS
Proximal tubular $(TF/P)_{In}$					
WKY	7–65	2.19	0.43	27	—
SHR	7–58	2.16	0.40	23	NS
	65	1.82	0.59	3	NS

[a] Data from anesthetized rats during micropuncture experiments.

rates, effective renal plasma flow (C_{PAH}), filtration fractions (FF), and proximal tubule $(TF/P)_{In}$ for the control and hypertensive groups. These measurements of renal function did not change with age in the normotensive series. Glomerular filtration rate was significantly depressed in SHR at week 65. There was a tendency for C_{PAH} to be significantly less ($P < 0.005$) in the SHR (weeks 7 to 58), which made for a larger filtration fraction in that population. The mean proximal $(TF/P)_{In}$ ratio was similar in both strains and did not vary with age.

Urinary excretion of protein. The excretion rates for total urinary protein in the SHR and WKY series are plotted against age in Figure 3a. The mean excre-

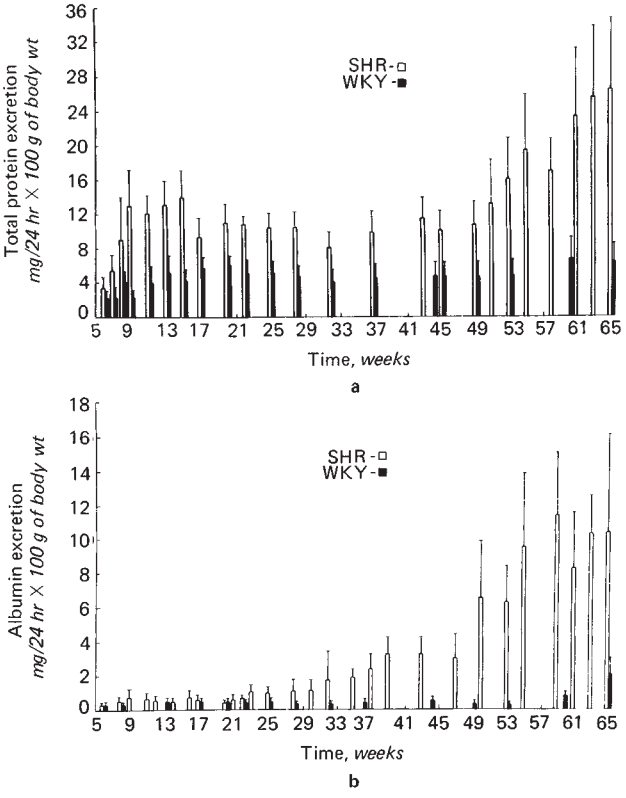


Fig. 3. *Protein excretion rate vs. age:* **a,** *total protein;* **b,** *albumin excretion.* The *vertical T-bars* denote SD. WKY, $N = 16$; SHR, $N = 20$.

tion rate in WKY controls for the entire period was 4.28 ± 0.95 (SD) mg/24 hr × 100 g of body wt. SHR's were found to have higher excretion rates at every age than the corresponding WKY strain, a difference that was more marked after the 45th week. At week 65, the protein excretion of the SHR's reached 26.6 ± 0.82 (SD) mg/24 hr × 100 g of body wt, six times the control value.

Urinary albumin excretion rates are plotted similarly to the above in Fig. 3b. In WKY controls,

albumin excretion was constant over the period of observation, the slight increase in the final weeks having been due to one animal. In SHR's, albumin excretion became significantly increased over WKY's at week 22, the difference widening rapidly thereafter (Fig. 3b). Albumin clearances, measured during micropuncture experiments, reached values six times that of normotensive controls (Table 3).

Table 3. Albumin excretion, clearance, and proximal tubular fluid concentration in WKY and SHR rats[a]

	Weeks	Mean	SD	No. of rats	*P*
Albumin excretion, *mg/24 hr × g of kidney wt*					
WKY	6–65	1.37	0.83	10	—
SHR	7–40	2.23	1.61	7	NS
	46–65	8.50	9.32	13	<0.025
Albumin clearance, *ml/min × g of kidney wt × 10^5*					
WKY	6–65	2.58	2.58	17	—
SHR	7–40	7.08	3.59	6	<0.025
	46–65	14.72	8.48	12	<0.001
Albumin clearance/GFR × 100					
WKY	6–65	0.006	0.007	12	—
SHR	7–40	0.016	0.009	5	NS
	46–65	0.029	0.013	11	<0.001
Proximal tubular fluid albumin, *mg/100 ml*					
WKY	6–65	2.63	0.92	28	—
SHR	7–40	2.88	1.43	17	NS
	40–65	2.10	1.82	14	NS

[a] Data from anesthetized rats during micropuncture experiments.

Electrophoretic patterns of urinary proteins are displayed graphically in Figure 4. The increasing importance of the albumin fraction after week 22 in SHR's is apparent. In young rats of both strains, low molecular weight (LMW) proteins (mobility > albumin) comprised the greater proportion of urinary proteins. As shown in Fig. 5b, this proportion was maintained in control rats throughout the period of study, but fell progressively in SHR's after week 30, coincident with the increase in albumin excretion in that strain (Fig. 5, a and b). At week 65, the fractional contribution of LMW proteins had fallen to less than 10% of total urinary protein in SHR's, and the absolute excretion rate of these LMW proteins was half the value in controls (2 vs. 4 mg/24 hr × 100 g of body wt). The percentage of globulins rose terminally in the SHR group (weeks 53–65) and appeared depressed in controls (Fig. 5c).

Tubular fluid albumin concentration. Albumin concentration in proximal tubule fluid samples, taken at random from surface nephrons of both SHR and WKY rats, did not reveal a systematic increment with age paralleling the increase in urinary albumin excretion (Table 3). There was no significant correlation (r = 0.34 or less) between tubular fluid albumin concentration and distance along the proximal tubule ($[TF/P]_{In}$) in either strain, or at any age.

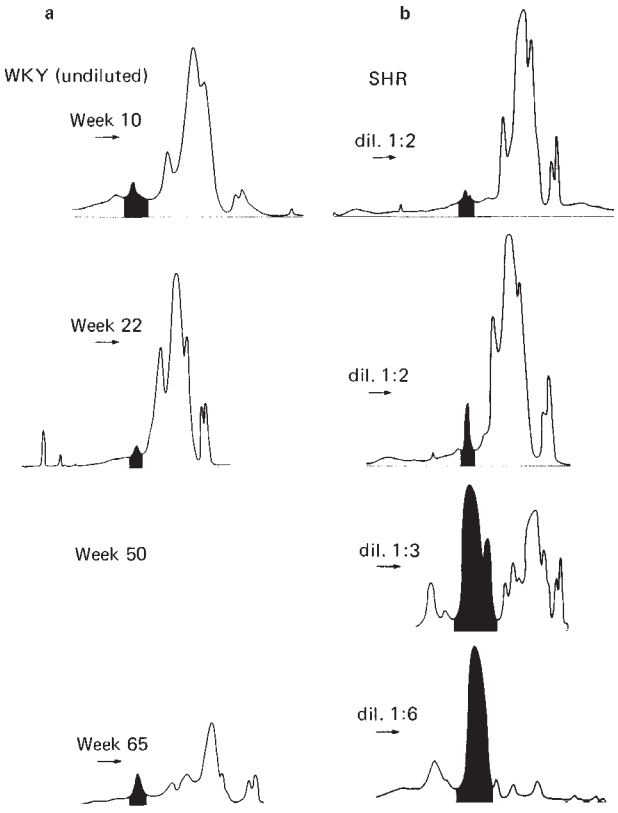


Fig. 4. *Urinary protein electrophoresis at various ages:* **a,** *WKY:* **b,** *SHR.* The *shaded area* is the albumin of each electrophoretic pattern.

Light microscopy of kidney and heart. The light microscopic study of the WKY strain showed no abnormalities of any nature from 6 to 65 weeks of age (Table 4). The SHR strain was not different from the WKY in the 7 to 20 week group, but a progressive increase in pathologic lesions was observed from week 30 on. These changes involved the glomeruli, tubules, interstitium, and arterial vessels of the kidney. Abnormalities in the heart were not observed until 46 weeks and were confined to the small arteries.

The 30 to 40 week group of hypertensive animals showed a moderate number of protein casts within the tubules of the inner one-third of the kidney cortex, (Table 4). The occurrence of casts preceded histologic glomerular changes. In the 30 to 40 week group, there was some increase in PAS-positive material surrounding Bowman's capsule. There were moderate lymphocytic infiltrations near venules at the affected glomeruli and tubules.

In the 46 to 50 week group of hypertensive rats, an abundant number of casts were found, located in the

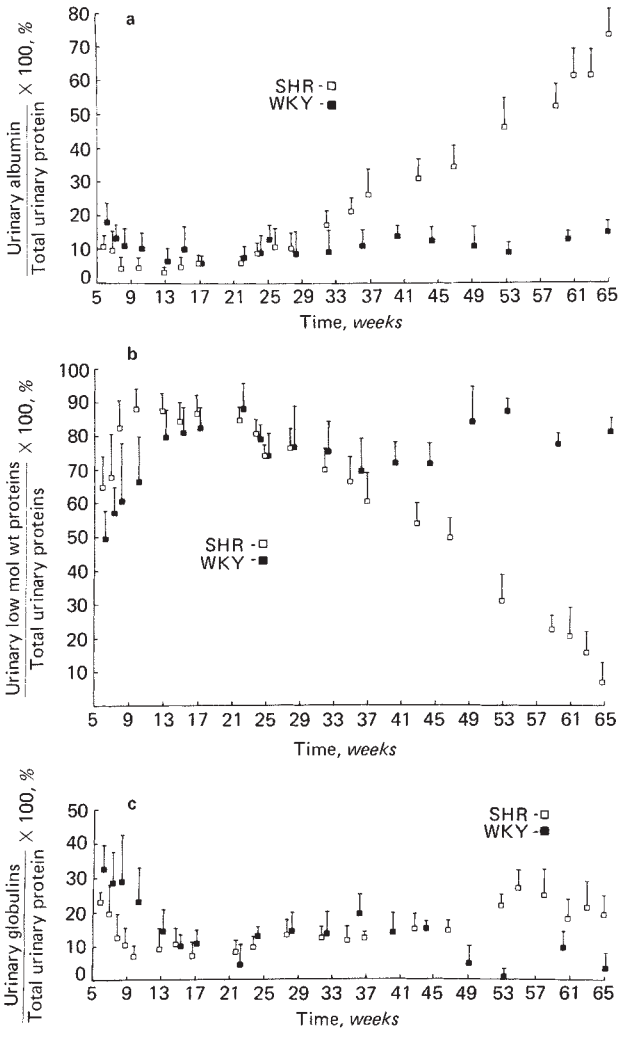


Fig. 5. *Fractional composition of urinary protein at various ages:* **a,** *albumin;* **b,** *low mol wt proteins;* **c,** *globulins.* The *vertical T-bars* denote SD. WKY, $N = 16$; SHR, $N = 20$.

inner two-thirds of the renal cortices. Some glomeruli were sclerotic, and hyaline material was present in both the axial and the peripheral location within the capillary tuft (Table 4). Glomeruli and vessels in the juxtamedullary zone were the first to be affected, and changes in this region were quite marked before the more superficial structures became involved. A sagital section through the cortex (Fig. 6a) demonstrates a gradient of severity in the glomerular lesions, increasing from superficial to deep. Fibrotic thickening of Bowman's capsule was quite pronounced in the PAS-stained sections (Fig. 6d).

The vascular lesions of the 46 to 50 week group were wide-spread, especially in the inner cortical zone (Fig. 7). These lesions included intimal proliferation, destruction of the internal elastic lamellae, duplication of the internal elastic lamellae, hyperplasia of medial smooth muscle cells, replacement of the smooth muscle cells by fibrinoid material, and obliteration of the vessel lumen.

All of the changes in the 46 to 50 week group were present in the 58 to 65 week group, but in greater severity (Table 4). Approximately 10% of the superficial tubules contained casts. These were not seen in any of the earlier age groupings. The distribution of the casts was focal, with the glomeruli in adjacent areas showing pronounced ischemic changes.

The vessels of the heart in the 46 to 50 and the 58 to 65 week groups showed similar lesions to those found in the kidney. Ballooning of the medial smooth muscle cells was found in most affected arteries.

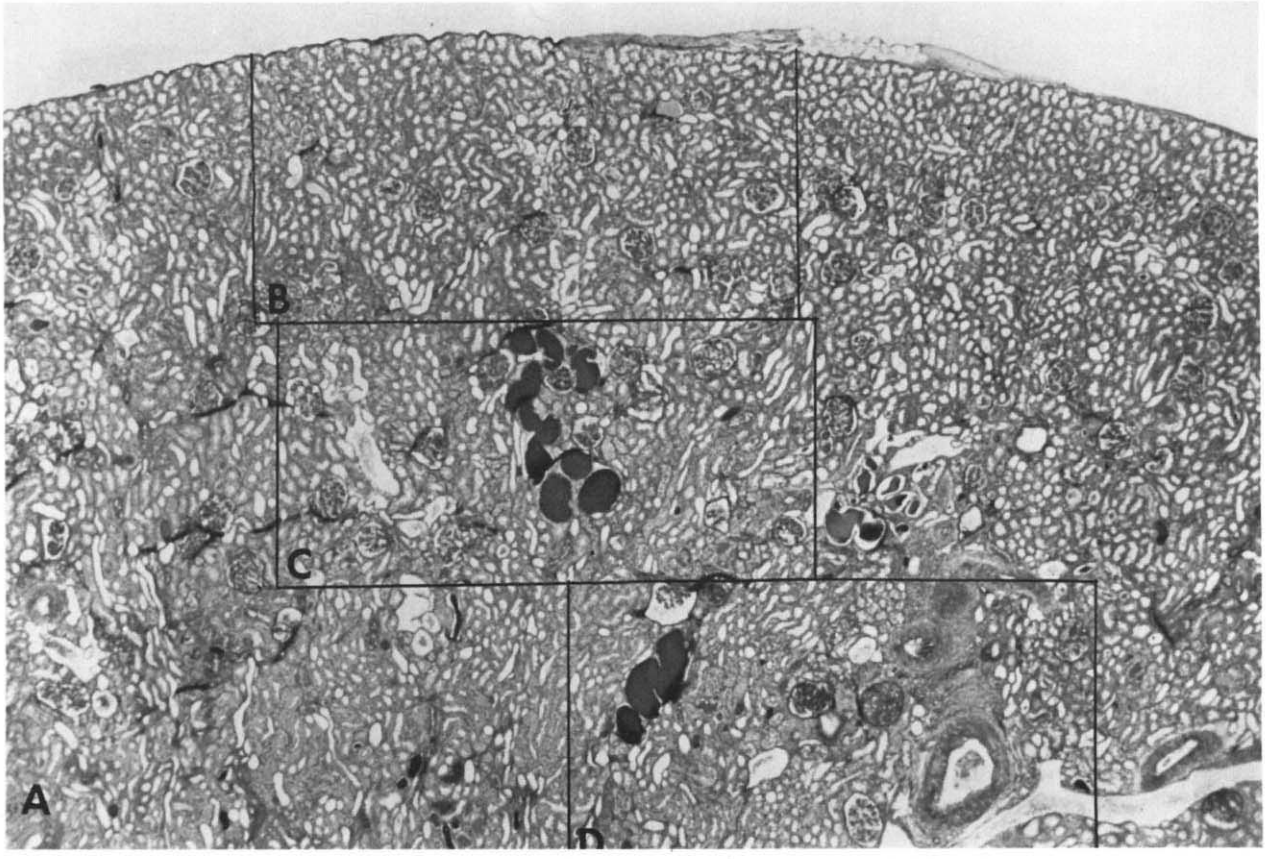
Discussion

It is generally accepted that the spontaneously hypertensive rat represents an analogue of human essential hypertension [14, 15]. The SHR strain

Table 4. Severity and location of renal cortical lesions[a]

Strain	Age,[a] weeks	Cortical zones[c]											
		Outer ⅓				Middle ⅓				Inner ⅓			
		0	+	++	+++	0	+	++	+++	0	+	++	+++
WKY	6–65 (27)	27	—	—	—	27	—	—	—	27	—	—	—
SHR	7–20 (12)	12	—	—	—	12	—	—	—	12	—	—	—
	30–40 (6)	6	—	—	—	—	1	1	—	—	6	1	—
	46–50 (8)	8	—	—	—	—	7	4	3	—	8	8	3
	58–65 (8)	7	1	—	—	—	8	5	4	—	8	8	6

[a] Classification of findings with light microscopy—
0 no lesions or casts
+ tubular casts
++ casts and moderate pericapsular fibrosis
+++ casts, marked fibrosis, sclerosis and/or obliteration of some glomeruli.
[b] Numbers in parentheses and in columns refer to numbers of animals.
[c] Zones defined by measurement with an eyepiece micrometer.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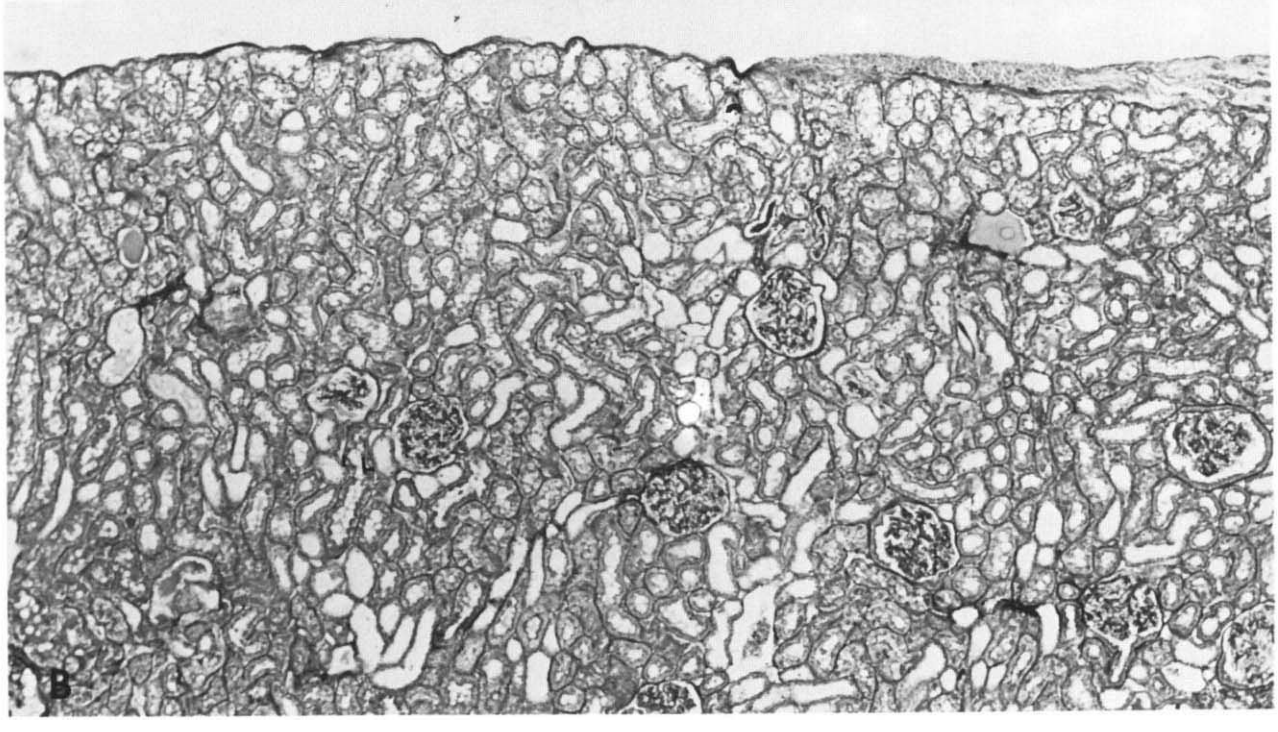
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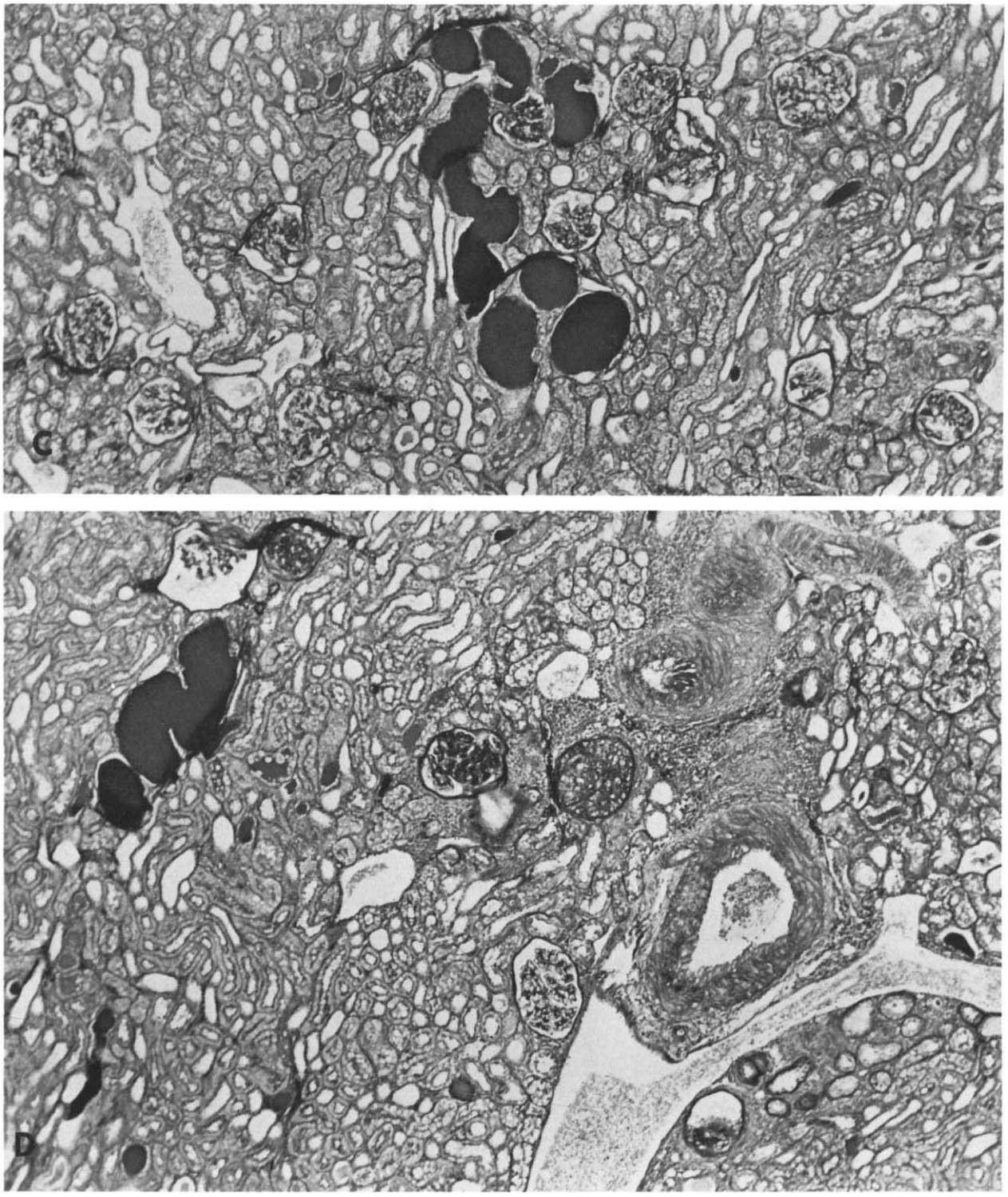

Fig. 6. A. *Sagital section of kidney cortex at 65 weeks in SHR.* There is a progressive increase in lesions as the photo is scanned from the superficial (top) to juxtamedullary zone (PAS stain: magnification, ×31). The framed areas are presented in higher magnification (×75) in B, C and D. **B.** *Outer ⅓ of cortex.* No lesions or casts are seen. **C.** *Middle ⅓ of cortex.* Large casts are seen in vicinity of glomeruli showing definite increase in PAS staining. **D.** *Inner ⅓ of cortex.* Pathologic changes are more pronounced. Pericapsular fibrosis is evident. Many glomeruli are damaged, several being markedly sclerotic. Small arteries show intimal proliferation, duplication of elastic lamina and muscular hyperplasia.

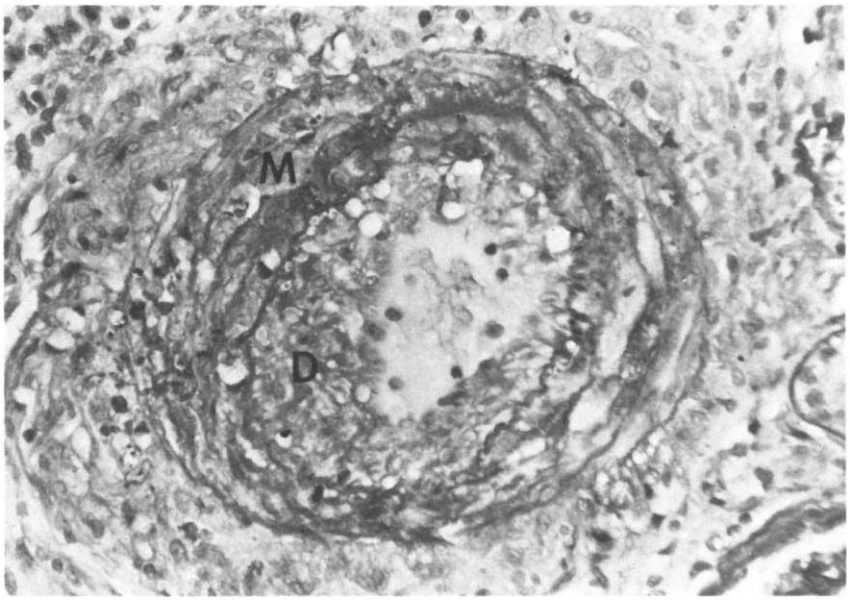

Fig. 7. *Small artery of the kidney at 46 weeks in SHR.* The vessel shows a crescent-shaped deposit **(D)** and proliferation compromising the lumen. The internal elastic lamina is destroyed at various sections of the vessel. The medial muscle layer has lost its architecture **(M).** PAS stain; magnification, ×530.

requires no dietary, surgical, or hormonal manipulations to attain chronic hypertensive levels [14]. Recent experiments by Yen et al [16] suggest that three to four genes control hypertension in the SHR, and at least one of these is a major gene.

Since the findings of Bell and Clawson [17] and Castleman and Smithwick [18], it is generally agreed that the vascular lesions associated with hypertension are the result, not the cause, of the increased pressure. Okamoto et al have described the characteristic vascular lesions of arteriolar nephrosclerosis in the SHR [19].

Except for the brief maturation period (weeks 9 to 13), the WKY controls tend to be slightly heavier than the SHR's. This difference was noted by Okamoto and Aoki in their initial report of the strain [14]. How much the weight difference is due to selective breeding, apart from the effects of hypertension, is not known. SHR's are said to be particularly susceptible to respiratory infections, but in our series both control and hypertensive groups seem equally affected.

No change in kidney weight per body weight is apparent in either strain within the time course of this study (Fig. 1). Cardiac hypertrophy in SHR (Fig. 1) is evident by the 30th week of life (17 weeks of hypertension), confirming the findings of others [20, 21]. Pfeffer and Frohlich [22] have established that the increase in heart weight is as in hypertensive man, predominantly due to an increase in thickness of the left ventricle.

Blood pressures are remarkable for the heights of the diastolic and mean arterial values. Representative values for average diastolic and mean arterial pressures are 3 and 2.5 times the respective control values. By human standards these animals would be considered as being in the malignant phase of hypertensive disease. An increase in heart rate from six to seven beats per second in the SHR contributes to the proportionate rise in diastolic pressure, resulting in similar pulse pressures in the hypertensive and normotensive groups. The mean of systolic blood pressures found in this study (199 mm Hg; Fig. 2) compares closely to that reported by other investigators [23]. Values obtained by direct arterial catheterization are similar to recent reports of others [22, 24, 25].

Creatinine clearance falls significantly in SHR after the 50th week (Table 1), and serum creatinine concentration rises. This probably represents a true fall in glomerular filtration rate, borne out by the smaller series of inulin clearances at comparable time periods (Table 2). The finding of a reduced renal plasma flow and normal filtration rate in the early stages of human essential hypertension has been recognized since the original studies of Goldring et al [26] and appears to hold for the SHR as well (Table 2) [27]. Whether the later fall in creatinine clearance is due to a reduction in filtration area from sclerotic change, or to a decrease in the hydraulic permeabili-

ty coefficient of the glomerular membranes cannot be determined from the present study.

Urinary excretion of total protein in SHR exceeds control rates at every age studied (Fig. 3a). Protein excretion in the WKY controls is indistinguishable from that observed in previous studies from this laboratory using domestic Wistar strains [11, 28]. No age-related proteinuria such as has been described in Sprague-Dawley rats [29] is seen in these Wistar strains. Inspection of Figs. 3b, 4b, 5a, and 5c reveals that the accelerated loss of urinary protein in SHR after the 45th week is due to a rapid increase in albumin excretion during this period and the appearance of a greater fraction of globulins in weeks 53 to 65. This protein excretion pattern is distinctive for the hypertensive strain, and its evolution becomes apparent after the 8th month of life.

Although there are small quantitative differences, the patterns of urinary protein in WKY and SHR are quite similar from weeks 5–30 (Figs. 3a through 5c). Both show an increase in the percentage of low molecular weight proteins during maturation (5 to 13 weeks) and a fall in the percentage of albumin and globulin. The effect of maturation on protein excretion, especially in the male rat, has been confirmed repeatedly [30–32] since it was first reported by Addis in 1931 [33]. Little change is noted in the absolute albumin excretion rate in either strain during this period (Fig. 3b). The modest but significant excess of total protein excretion in SHR over WKY during maturation (Fig. 3a) is probably not related to the effects of hypertension, since it is manifest before hypertension is established and exhibits an extended period of stability (weeks 13 to 49) during which no further increase in protein excretion occurs in either strain. The predominance of albumin in the subsequent proteinuria characteristic of SHR was reported briefly by Nagaoka et al [34]. The increase in urinary globulins during the final weeks of observation (Fig. 5c) documents a diminished selectivity in the defect of the filtration barrier.

Low molecular weight proteins constitute an important fraction of the total urinary protein in the rat (Fig. 5b), a finding first appreciated by Finlayson and Morris [35]. The percentage of LMW proteins rises sharply during maturation in both SHR and WKY controls, confirming an earlier report from this laboratory [36]. These elevated levels of both fractional and absolute excretion are sustained in the control group, but both fall steadily after the 30th week in SHR, corresponding to the drop in creatinine clearance (Fig. 5b, Table 1). This coincidence may be expected because a reduced filtration area restricts the access of filterable molecules to excretion. Selective permeability changes may also be a factor. The phenomenon of increased permeability to large molecules ($\geqslant$alb), while that to smaller macromolecules may be actually diminished, is characteristic also of angiotensin-induced proteinuria [11], minimal change nephrotic syndrome [37], and some cases of proliferative glomerulonephritis [38].

Although urinary albumin excretion in SHR's at weeks 46 to 65 has increased many fold over the value in controls, tubular fluid albumin concentration in surface nephrons is indistinguishable in the two groups (Table 3). As in the normal domestic Wistar rat, there is no correlation between tubule fluid albumin concentration and proximal fluid reabsorption ($[TF/P]_{In}$). The significance of this finding is discussed in our earlier publications [4, 11, 28]. Nephrons with surface representation account for approximately 50% of the total nephron population in the rat [39, 40] and the absence of change in TF_{alb} in these loops suggests a major contribution of the deeper glomeruli to albumin excretion. This thesis finds support in the selective localization of pathology in our SHR's, as well as in anatomical and hemodynamic differences in juxtamedullary versus superficial nephrons.

Juxtamedullary glomeruli are the site of initial protein leakage, as evidenced by the appearance of casts in adjacent tubules as early as the 30th week (Table 4). At the 46th week, glomerular lesions were apparent by light microscopy. The changes of hyalinization, pericapsular fibrosis, and round cell infiltration and obliteration of Bowman's space increase in severity as sections are scanned from outer cortex to juxtamedullary zone (Fig. 6). A similar gradient of involvement was found in small arteries and arterioles, showing the classic changes of intimal proliferation, duplication of elastic lamina, medial hyperplasia, and fibrinoid degeneration (Fig. 7) which was most marked in the deep cortical structures. Kidneys of WKY controls show no pathologic lesions at any age studied. This is in marked contrast to the findings of Couser and Stillman [29] in Sprague-Dawley rats, a strain notable for the development of age-related proteinuria, focal glomerular sclerosis, and mesangial lesions.

Juxtamedullary glomeruli have distinctive characteristics. They are larger than their superficial counterparts [41, 42], and their efferent arterioles are of larger diameter [42, 43]. Positioned at the root of the interlobular arteries from which their afferents take origin, the juxtamedullary and inner cortical glomeruli are exposed to the highest pressures. Jamison et al have calculated that an excess of even 30 mm Hg of juxtamedullary over superficial net filtration pres-

sure is insufficient to account for the larger filtration rate of juxtamedullary glomeruli, given the filtration areas determined from histologic measurements [44]. The medullary blood flow, derived from deep cortical efferent arterioles, is not autoregulated but increases with increments in renal artery pressure [45, 46]. These indirect lines of evidence are consistent with a primary effect of hypertension on juxtamedullary glomeruli.

These considerations are supported by the experimental findings of Stumpe, Lowitz, and Ochwadt [47] who compared single nephron filtration rates (SNGFR) of superficial and deep nephrons in normal and hypertensive (two-kidney Goldblatt) rats. Juxtamedullary SNGFR's were proportional to the increase in arterial blood pressure, whereas superficial values were constant (regulated) to pressures up to 200 mm Hg. Azar, Johnson, and Tobian [27] have recently reported equal SNGFR's and glomerular capillary pressure in surface nephrons of WKY and SHR at 18 weeks of age, confirming autoregulation in the latter strain. Filtration rates of superficial and deep nephrons in the SHR have been reported by Farman and Bonvalet [48] and were found to be not different from normotensive series in the literature. Since these authors were working with a younger group of SHR's, having a significantly lower mean arterial pressure (165 vs. 192.8 mm Hg) than animals in the present study, the question of autoregulation in juxtamedullary nephrons is still unanswered.

Although we have not excluded a biochemical or structural defect in the filtering membrane of the SHR kidney, possibly linked genetically to the determinants of its hypertension, the evidence is consistent with the following conclusions: *1)* a distinguishing pattern of proteinuria evolves during the first year of life in the spontaneously hypertensive rat, *2)* the pattern is characterized by an increase in albumin and a decrease in low molecular weight protein excretion by the kidneys, *3)* evolution of the pattern of protein excretion is related temporally to the appearance of glomerular and arteriolarsclerotic change and a fall in creatinine clearance, *4)* pathologic changes appear first and progress more rapidly in the juxtamedullary and deep cortical glomeruli and small vessels, and *5)* anatomical and hemodynamic (non-autoregulatory) factors may be responsible for this localized predilection to injury.

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